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INHIBITION OF DIHYDROPTERIDINE REDUCTASE IN RAT STRIATAL SYNAPTOSOMES AND FROM HUMAN LIVER BY METABOLITES OF BIOGENIC AMINES

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Catecholamines are potent noncompetitive inhibitors of dihydropteridine reductase in rat striatal synaptosomal preparations or purified from human liver. Their metabolities, except homovanillic acid, also inhibit the enzyme from both sources. The inhibitory potency of these compounds depends on the presence of the catechol or the 4-hydroxyphenyl structure, but may be modified by the 2-carbon side chain and its substituents. Indoleamines which have a hydroxylated aromatic nucleus (5-hydroxytryptamine and 5,6-dihydroxytryptamine) are equally inhibitory to the enzyme. These results suggest that biogenic amines themselves rather than their metabolities may serve as physiological inhibitors of dihydropteridine reductase in rat brain.

KEY WORDS: Dihydropteridine reductase; biogenic amines and their metabolites.

ABBREVIATIONS: MOPET, 4-Hydroxy-3-methoxyphenethyl alcohol; DOPAC, 3,4-Dihydroxyphenylacetic acid; HVA, 4-Hydroxy-3-methoxyphenylacetic acid; DOPEG, DL-3,4-Dihydroxyphenylglycol; MOPEG, DL-4-Hydroxy-3-methoxyphenylglycol; DOMA, DL-3,4-Dihydroxymandelic acid; VMA, DL-4-Hydroxy-3-methoxymandelic acid; $DMPH_4$, 2-Amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine; 5HIAA, 5-Hydroxyindole-3-acetic acid; $qDMPH_2$, Quinonoid 2-amino-6,7-dimethyl-4-hydroxydihydropteridine.

Catechol- or *p*-hydroxyphenyl-containing compounds, such as catecholamines[1,2], dopamine-derived tetrahydroisoquinolines[3], catechol estrogens[4], tyrosine metabolites[5], 4-hydroxyphenyl-1,2,3,6-tetrahydropyridines[6], hydroxylated nomifensines[7], and aporphines[8] are known inhibitors of dihydropteridine reductase (E.C. 1.6.99.7) (NADH : 6,7-dihydropteridine oxidoreductase). This enzyme regenerates tetrahydrobiopterin from quinonoid dihydrobiopterin for the hydroxylation of phenylalanine, tyrosine, and tryptophan during biogenic amine synthesis[9]. Inhibition of the activity of dihydropteridine reductase could eventually deplete the synthetic and catalytic pool of the biopterin cofactor, which in turn could limit neurotransmitter synthesis and render tyrosine 3-hydroxylase (E.C. 1.14.16.2) more sensitive to end-product inhibition.

Since dopamine and norepinephrine metabolites retain their catechol or *p*-hydroxyphenolic moiety, one purpose of this study was to examine whether these compounds are effective inhibitors of rat striatal and human liver dihydropteridine reductase. The inhibitory effectiveness of indoleamines and their metabolites as inhibitors of the human liver enzyme was also examined.

MATERIALS AND METHODS

Materials

The following compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI): dopamine (3,4-dihydroxyphenylethylamine) hydrochloride, 3-*O*-methyldopamine (3-methoxytyramine) hydrochloride, 4-hydroxy-3-methoxy-phenethyl alcohol (MOPET, homovanillyl alcohol), 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (HVA, homovanillic acid), (−)-norepinephrine hydrochloride, DL-3,4-dihydroxyphenylglycol (DOPEG), DL-4-hydroxy-3-methoxyphenylglycol (MOPEG, piperazine salt), DL-3,4-dihydroxymandelic acid (DOMA), DL-4-hydroxy-3-methoxymandelic acid (VMA, DL-vanillomandelic acid, (−)-epinephrine, L-tryptophan, tryptamine hydrochloride, and 2-amino-6,7-dimethyl- 4-hydroxy-5,6,7,8-tetrahydropteridine ($DMPH_4$) hydrochloride. The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): DL-3-*O*-methylnorepinephrine (DL-normetanephrine) hydrochloride, DL-3-*O*-methylepinephrine (DL-metanephrine) hydrochloride, *l*-5-hydroxytryptophan, serotonin (5-hydroxytryptamine, creatinine sulfate monohydrate), 5,6-dihydroxytryptamine (creatinine sulfate), 5-methoxytryptamine hydrochloride, 5-hydroxyindole, 5-hydroxyindole-3- acetic acid (5HIAA), DL-indole-3-lactic acid, indole-3-pyruvic acid, tryptophol, horse- radish peroxidase, and NADH. Solutions of these compounds were prepared fresh in 0.05 M Tris-HCl buffer (pH 6.8) and degassed immediately prior to use.

Preparation of Synaptosomal Suspension

Rat striatal synaptosomes were prepared by the method of Gray and Whittaker[10] with slight modification[8]. Male Sprague–Dawley rats (220–280 g) were decapitated and their striatal tissue dissected out and homogenized in 10 ml of 0.32 M sucrose solution (pH 7.4) with a motor-driven teflon pestle-glass homogenizer (0.13 to 1.8 mm clearance). Striatal homogenates were centrifuged at 1000 × *g* for 15 min. The resulting supernatant was centrifuged at 17,500 × *g* for 20 min to sediment the P_2 fraction, which was finally resuspended in 15 vol (w/v) of a buffered medium containing: 50 mM Tris-HCl (pH 7.4), 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose. Dihydropteridine reductase activity in synaptosomes was assayed at 50 μM of each substrate. Kinetics studies indicated dihydropteridine reductase in rat striatal synaptosomes had K_m values of 17 μM for $qDMPH_2$ and 10 μM for NADH.

Human Liver Dihydropteridine Reductase

The enzyme was purified by ammonium sulfate precipitation, sequential column chromatography on DEAE-Sephacel, Matrex Gel Blue A, and hydroxyapatite, and high performance liquid chromatography, according to the procedures described previously[3,11]. The enzyme preparation used in this study was form BII whose physical and kinetic properties have been described[2].

Determination of Dihydropteridine Reductase Activity

The enzyme activity was determined spectrophotometrically by measuring the rate of

TABLE I

K_i and I_{50} values of catecholamine metabolites as inhibitors of dihydropteridine reductase. Rat striatal synaptosomes (6.5 milliunits or 100 μg P_2 protein) or human liver enzyme (6 milliunits or 60 ng protein) was incubated 10 min with each compound at 25°C. Residual enzyme activity was assayed at 50 μM of each substrate to obtain I_{50} values, and at different $qDMPH_2$ concentrations (20 to 50 μM) while the concentration of NADH was kept constant (50 μM), to obtain K_i values. All values are single or the average of two determinations, and are expressed in M.

Compound	Rat striatal synaptosomes		Human liver	
	I_{50}	K_i	I_{50}	K_i
Dopamine	2.4×10^{-5}	1.3×10^{-5}	2.0×10^{-5}[a]	1.4×10^{-5}[a]
3-*O*-methyldopamine	5.0×10^{-5}		6.0×10^{-5}[a]	
4-Hydroxy-3-methoxyphenethyl alcohol	1.4×10^{-5}		1.4×10^{-5}	
3,4-Dihydroxyphenylacetic acid	7.2×10^{-5}	8.1×10^{-5}	6.7×10^{-5}	9.0×10^{-5}
4-Hydroxy-3-methoxyphenylacetic acid	3.1×10^{-3}		1.8×10^{-3}	
(−)-Norepinephrine	1.4×10^{-4}		2.0×10^{-4}[a]	1.9×10^{-4}[a]
DL-3-*O*-Methylnorepinephrine	1.2×10^{-4}		1.1×10^{-4}	
DL-3,4-Dihydroxyphenylglycol	1.9×10^{-6}	1.4×10^{-6}	9.1×10^{-6}	9.6×10^{-6}
DL-4-Hydroxy-3-methoxyphenylglycol	1.7×10^{-5}		1.9×10^{-5}	
DL-3,4-Dihydroxymandelic acid	1.3×10^{-5}	1.7×10^{-5}	8.5×10^{-6}	9.0×10^{-6}
DL-4-Hydroxy-3-methoxymandelic acid	1.2×10^{-4}		1.9×10^{-4}	
(−)-Epinephrine	9.0×10^{-5}		1.3×10^{-4}[a]	1.1×10^{-4}[a]
DL-3-*O*-Methylepinephrine	1.5×10^{-4}		1.5×10^{-4}	

[a]Data taken from Ref. 2 for comparison.

disappearance of NADH at 340 nm at 25°C, according to the method of Nielson *et al.*[12] The quinonoid $DMPH_2$ was generated *in situ* by the peroxidase-catalyzed oxidation of $DMPH_4$ in the presence of H_2O_2.

Enzyme Inhibition

The reaction mixture in the presence and absence of inhibitors has been described[3,4]. Methods for obtaining the dissociation constants of the enzyme-inhibitor complexes (K_i values) and the inhibitor concentrations that give 50% inhibition of the enzyme activity (I_{50} values) have also been described[2].

RESULTS

Table I summarizes I_{50} and K_i values of catecholamines and their metabolites as inhibitors of dihydropteridine reductase either contained in rat striatal synaptosomes or purified from human liver. The use of synaptosomes as an enzyme source allowed the examination of the inhibition of dihydropteridine reductase activity in the presence of other enzymes and cofactors which are involved in dopamine synthesis. The I_{50} values obtained for these compounds against dihydropteridine reductase from both sources were approximately the same. This similarity suggests that the molecular structures of the enzyme in both species are related.

All the catecholamines and their metabolites except HVA were effective inhibitors of this enzyme with I_{50} values in the range of 10^{-4} to 10^{-6} M (HVA, I_{50} = 1.8 to 3.1×10^{-3} M). The K_i values obtained for 6 compounds against dihydropteridine reductase from both sources paralleled their respective I_{50} values. These relationships

TABLE II

K_i and I_{50} values of indoleamines and their precursors and metabolites as inhibitors of human liver dihydropteridine reductase. Human liver dihydropteridine reductase (6 milliunits or 60 ng protein) was incubated 10 min with each compound at 25°C. Residual enzyme activity was assayed at 50 μM of each substrate to obtain I_{50} values, and at different $qDMPH_2$ concentrations (20 to 50 μM) while the concentration of NADH was kept constant (50 μM), to obtain K_i values. All values are single or the average of two determinations

Compound	I_{50} (M)	K_i (M)
L-Tryptophan	$> 1.0 \times 10^{-2}$	
l-5-Hydroxytryptophan	6.1×10^{-3}	
Serotonin	4.2×10^{-5}	4.8×10^{-5}
5,6-Dihydroxytryptamine	1.1×10^{-5}	9.1×10^{-6}
Tryptamine	$> 5.0 \times 10^{-3}$	
5-Methoxytryptamine	$> 1.0 \times 10^{-2}$	
5-Hydroxyindole	1.5×10^{-4}	
5-Hydroxyindole-3-acetic acid	1.1×10^{-3}	
DL-Indole-3-lactic acid	3.7×10^{-3}	
Indole-3-pyruvic acid	3.7×10^{-4}	
Tryptophol	9.6×10^{-4}	

suggest a noncompetitive type of inhibition[13]. Lineweaver–Burk plots of the inhibition kinetics of the enzyme in the presence of these compounds (data not shown, see Ref. 2) confirm this conclusion, which is consistent with results obtained previously[2-8].

Table II summarizes I_{50} and K_i values of indoleamines and their precursors and metabolites as inhibitors of human liver dihydropteridine reductase. L-Tryptophan and 5-methoxytryptamine did not inhibit dihydropteridine reductase ($I_{50} > 10$ mM). *l*-5-Hydroxytryptophan, tryptamine, 5-HIAA, and DL-indole-3-lactic acid were weakly effective inhibitors of this enzyme ($I_{50} > 1$ mM). However, 5-hydroxyindole, indole-3-pyruvic acid, and tryptophol were effective inhibitors ($I_{50} = 1.1$ to 9.6×10^{-4} M), whereas serotonin and 5,6-dihydroxy-tryptamine were potent inhibitors of dihydropteridine reductase with K_i values of 4.8×10^{-5} M and 9.1×10^{-6} M, respectively.

DISCUSSION

The inhibitory potency of dopamine metabolites are approximately the same as dopamine, whereas norepinephrine metabolites are generally more potent than norepinephrine. It has been found that the conversion of dopamine to norepinephrine through beta-hydroxylation of the 2-carbon side chain reduces the enzyme inhibitory potency by 10-fold[2]. The results of this study indicate that deaminated norepinephrine metabolites regain their enzyme inhibitory potency. Thus the I_{50} values of DOPEG, DOMA, and MOPEG are comparable with that of dopamine. It appears, therefore, that intraneuronal oxidation of dopamine and norepinephrine by monoamine oxidase (E.C. 1.4.3.4), followed by alcohol dehydrogenase (E.C. 1.1.1.1) and aldehyde dehydrogenase (E.C. 1.2.1.3), does not alter the inhibitory potency of these neurotransmitters as inhibitors of dihydropteridine reductase.

Methylation of acidic catechols by extraneuronal catechol-*O*-methyltransferase (E.C. 2.1.1.6) greatly reduced their inhibitory potency against dihydropteridine

reductase. I_{50} values of HVA and VMA were 10- to 40-fold higher than those of their immediate precursors, DOPAC and DOMA, respectively. Methylation of basic and neutral catechols, such as dopamine, norepinephrine, epinephrine, and DOPEG, however, did not alter their I_{50} values to such a great extent (only 0.5- to 3-fold reduction). The 2-carbon side chain and its substituents, especially the ionized group, may play a role in the inhibition of dihydropteridine reductase[2], but the mechanism of this reaction is unclear.

Like other catechols, 5,6-dihydroxytryptamine is a potent inhibitor of human liver dihydropteridine reductase. However, other indoles, except 5-hydroxylated compounds such as serotonin and 5-hydroxyindole, are ineffective inhibitors. These results demonstrate that the catechol moiety of the indole ring is still inhibitory to dihydropteridine reductase, like the catechol nuclei which are essential for the inhibition of dihydropteridine reductase by multiple ring compound such as tetrahydroisoquinolines[3], catechol estrogens[4], and aporphines[8].

Purdy and Blair reported that rat liver dihydropteridine reductase was inhibited competitively by serotonin and in mixed fashion by tryptophan[14]. Moreover, in comparison with the data presented here, their values for the apparent K_i value for serotonin (1.2×10^{-3} M) is approximately 25 times higher, and that for tryptophan (2.0×10^{-4} M) is at least 50 times lower. These disagreements cannot be readily explained; they probably do not arise from the use here of enzyme from different species, because when tested with phenylalanine and phenylpyruvate no difference was found in I_{50} values between the enzyme obtained from rat and human liver[5].

Armarego and Waring reported that *in vitro* inhibition of dihydropteridine reductase by dopamine was due to its oxidative products[15]. However, their enzyme assay conditions differed greatly from those used here in that they incubated dopamine with peroxidase, H_2O_2, pterin substrate, and NADH prior to the additon of the enzyme, whereas we pre-incubated dopamine with the enzyme for 10 min, followed by the addition of the rest of reagents to assay the residual enzyme activity against a no-enzyme blank[3,4]. Besides, our enzyme assays usually last only 2 min, and we observe no deviation in absorbance at 340 nm when the assay is allowed to continue for an additional 2 to 4 min. Although the present study does not define the mechanism of inhibition of dihydropteridine reductase by dopamine and other catechol-containing compounds, we have previously proposed that the catechol group may undergo quinone formation, and then attack a nucleophile in the enzyme molecule[4]. The finding of Armarego and Waring[15] that aminochromes, which contain quinone structures, are potent inhibitors of dihydropteridine reductase supports our hypothesis.

The levorotatory isomers of norepinephrine and epinephrine, but the DL-racemates of their metabolites, were tested in this study. If the inhibition of dihydropteridine reductase by these compounds was stereospecific for levo-isomers, the I_{50} values obtained with the metabolites would be halved. We have found that the *S*-(+)-enantiomers of aporphines are as potent as their corresponding *R*-(−)-enantiomers[8], and 17 α-estradiol is as potent as 17 β-estradio[4] in inhibiting dihydropteridine reductase. Therefore, the inhibition of dihydropteridine reductase by biogenic amines and their metabolites may not be stereospecific, in which case the I_{50} values obtained with the DL-racemates would be equivalent to those obtained with the corresponding levorotatory isomers.

The major metabolites of dopamine, norepinephrine, and serotonin in cerebral

spinal fluid are HVA, MOPEG, and 5HIAA, respectively[16]. Other minor metabolites are MOPET, 3,4-dihydroxyphenethyl alcohol, and DOPAC for dopamine, VMA for norepinephrine, and indole-3-acetic acid for tryptamine. If cerebral spinal fluid levels of these metabolites reflect the rate of their production in the central nervous system, these metabolites of biogenic amines may not inhibit dihydropteridine reductase centrally, for HVA and 5HIAA are ineffective inhibitors of the enzyme ($I_{50} > 1$ mM), and the I_{50} value of MOPEG is approximately 200 times greater than its concentration in cerebral spinal fluid[17]. It is more likely that biogenic amines themselves, especially dopamine and serotonin, rather than their metabolites, serve as physiological inhibitors of dihydropteridine reductase in the central nervous system.

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